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Ultrasound Elastography to Determine the Layered Mechanical Properties of Articular Cartilage and the Importance of Such Structural Characteristics under Load

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Abstract— **Articular cartilage is an important load bearing surface in joints. Prone to damage and with limited self-repair ability, it is of interest to tissue engineers. Tissue implant design requires full mechanical characterisation of healthy native tissue. A layered organisation of reinforcing collagen fibrils exists in healthy articular cartilage and is believed to be important for correct tissue function. However, the effect of this on the local depth-dependent elasticity is poorly characterised. In this study, quasi-static ultrasound elastography is used both to compare the depth-dependent elastic properties of cartilage structures with two different fibril arrangements and to monitor changes in the elastic properties of engineered samples during development. Results show global and local elastic properties of the native tissues and highlight the differences caused by fibril architecture. At increasing culture periods, results from the engineered tissue demonstrate an increase in elastic stiffness and the time taken to reach equilibrium under a quasi-static displacement. The study suggests suitability of ultrasound elastography for design and monitoring engineered articular cartilage.**

I Introduction

Articular cartilage is an important load bearing tissue found in joints. It is a specialized structure of hyaline cartilage. Hyaline cartilage is a poroviscoelastic solid containing fibril matrix reinforcements. Healthy articular cartilage consists of three layers of differing elastic properties primarily conferred by the orientation of the fibril reinforcements in those regions. Collagen fibril arrangement in the layers of articular cartilage can be approximated as parallel to the surface in the superficial zone, perpendicular to the surface in the deep zone and random in the transitional (or middle) zone. This arrangement is shown in figure 1 [1]. The layered nature of articular cartilage is thought to be important for correct tissue function by allowing stresses to be distributed in such a way so as to avoid damage. However, the behaviour of each layer during loading is poorly characterised. Articular cartilage has shown limited regeneration ability following damage. In order for tissue engineers to design functional constructs full understanding of the properties of healthy tissue is required.

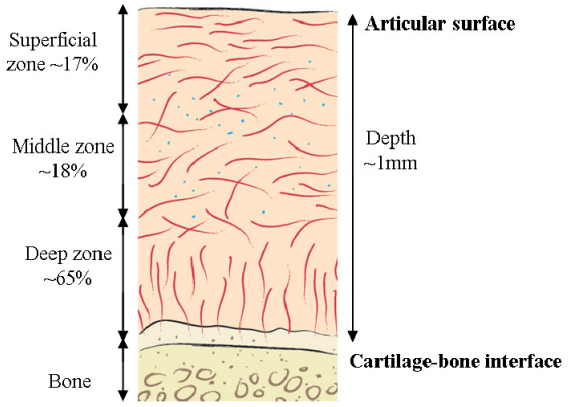

Figure 1: An approximation of the arrangement of collagen fibrils through a cross-section of articular cartilage [1]

Ultrasound elastography provides access to depth-dependent information in a sample during real-time loading protocols. The technique of elastography is based on the principle of tracking small shifts in signal arrival time to determine localised strains [2]. Through the application of elastography during quasi-static loading the depth-dependent strains within cartilage samples can be found. The depth-dependent strain data can be compared to global mechanical property measurements for the same samples and the elastic properties of the layers during the loading protocol can be found. Elastography techniques can also be applied to monitor the development of engineered tissue with respect to time. The information provides a more comprehensive assessment of the progress of the tissue culture than histology and conventional mechanical tests.

Elastographic and mechanical results for articular cartilage are compared to nasal cartilage which is a non-layered hyaline cartilage. Nasal cartilage has collagen arrangements similar to the middle zone of articular cartilage. Engineered cartilage tissue samples (collagen seeded with chondrocytes), at different developmental stages, are assessed in terms of their elastic properties and homogeneity.

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II Materials / Methods

A. Samples

Porcine nasal and bovine tibial articular cartilage samples were obtained from a local abattoir within 4 hours of slaughter and prepared into discs of 8mm diameter using biopsy punches. Once prepared the samples were stored in phosphate buffered saline solution (PBS) until testing. Ten samples of each cartilage type for each test were prepared. The samples were tested in a tank filled with PBS.

Chondrocyte cells were extracted from the same joint cartilage and cultured. These cells were later seeded onto dense collagen scaffolds [3] and cultured for periods of 5, 7 and 10 days. The samples at each culture period were prepared by stacking five cultured sheets (to provide a sample of suitable thickness to provide reasonable elastography data) and then sectioned into discs with a diameter of 8mm.

B. Mechanical Testing

An actuator was used to apply constant strains of 2, 4, 6 or 8% to the top surface of the samples, while the lower surface was against a fixed rigid surface (the ultrasonic delay-line). The sides of the disc were unconfined. The strains were held for 20 minutes. A load cell was used to measure the force required to deform the samples. A linear variable differential transformer (LVDT) was placed in parallel with the load cell to measure the small deformations for use in the analysis. To calculate the elastic modulus from the data the nominal strains applied to the samples were corrected using the data from the LVDT. A diagram of the complete equipment set-up is shown in figure 2.

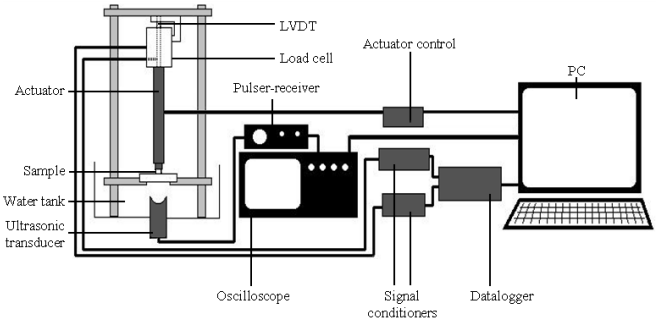

Figure 2: Schematic of the equipment set-up

C. Ultrasonic Testing

An ultrasound beam with a centre frequency of 20MHz was focussed through a delay-line into the samples. Focussed ultrasound was selected to allow data collection from only small regions in the centre of each sample. The ultrasonic A-lines were recorded at three-second intervals from before the strain application and during the constant strain period. The A-line signals before the strain application were compared to signals received immediately after the strain application and 10 and 20 minutes thereafter. The ultrasonic data were transformed into strains through a signal processing algorithm. The strain maps were created through plotting the strain versus depth.

The global results from the mechanical tests were linked to the ultrasonic data through the use of a constitutive equation based mechanical model which is optimised to provide localised elastic moduli from the depth-dependant strain data.

III Results

The mechanical results in figure 3 showed the instantaneous modulus of articular cartilage to be strain dependent, unlike the nasal cartilage test results. The stress-relaxation rates were seen to be higher at lower strain levels, particularly for articular cartilage. The equilibrium moduli were, however, similar for both cartilage types at all levels of applied strain (approximately 1.5MPa for nasal and 1.3MPa for articular).

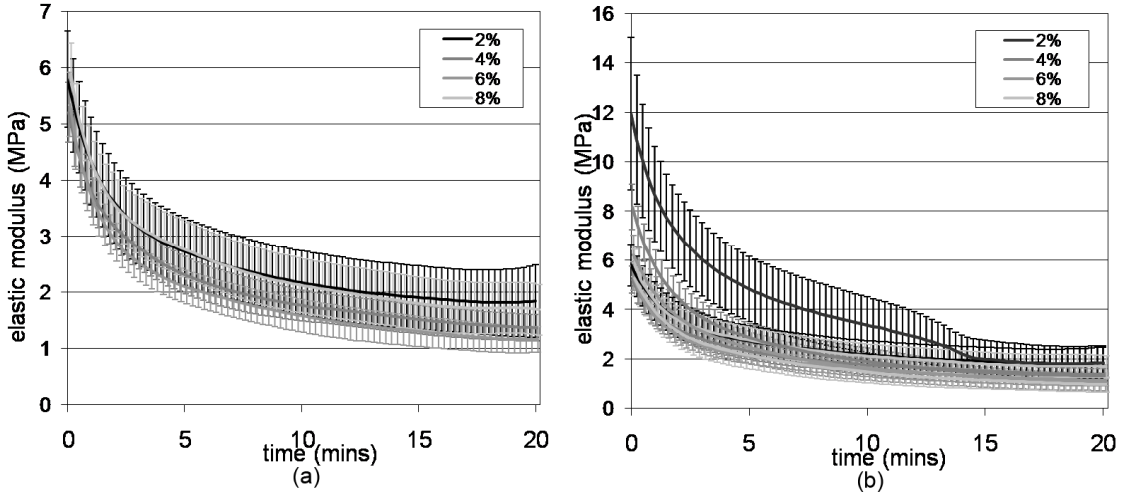

Figure 3: The stress-relaxation curves for (a) nasal and (b) articular cartilage samples over 20 minutes

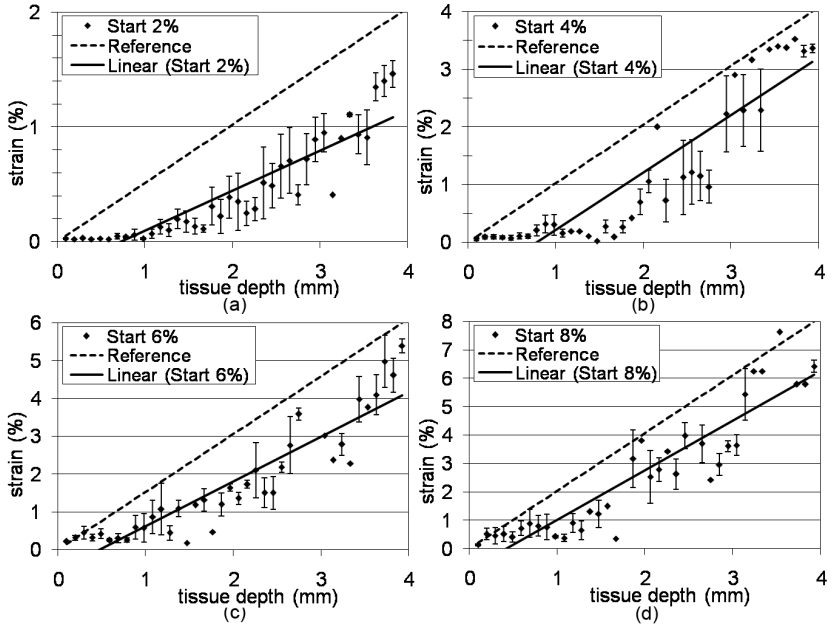

Figure 4: The elastography strain maps of internal scatterer shifts in nasal cartilage samples at (a) 2%, (b) 4%, (c) 6% and (d) 8% applied strain at the instant of load application

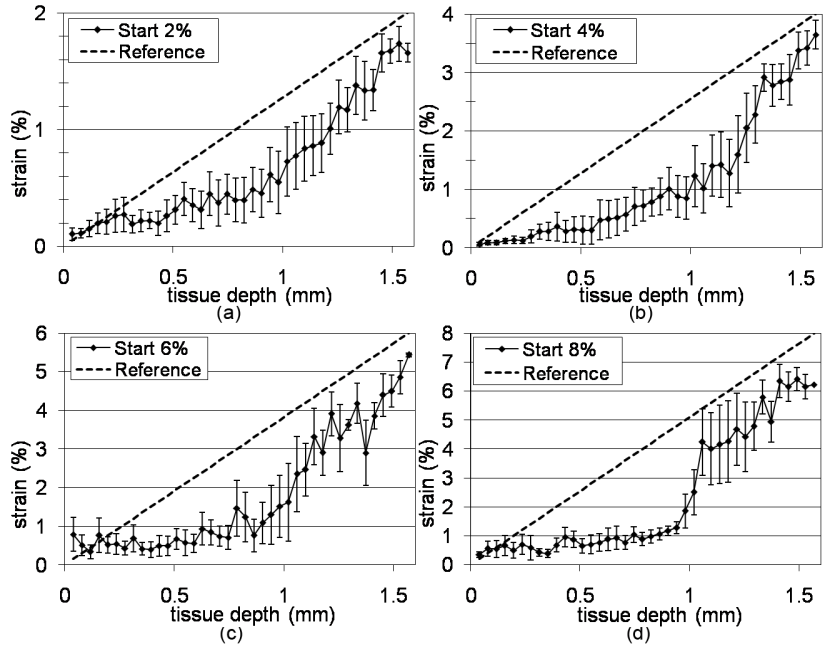

Figure 5: The elastography strain maps of internal scatterer shifts in articular cartilage samples at (a) 2%, (b) 4%, (c) 6% and (d) 8% applied strain at the instant of load application

The ultrasonic results showed that the global speed of sound propagation was higher through the articular samples than the nasal samples. The elastography strain maps found for the nasal cartilage in figure 4 showed roughly linear trends, whereas the articular cartilage strain maps in figure 5 showed evidence of different behaviour in separate regions. The differences between the regions increased at the instant of loading in articular cartilage with increasing strain. This strain dependent difference was no longer evident after 20 minutes of stress-relaxation.

The mechanical results from the engineered tissue indicated increasing stiffness with increasing culture time. The samples were seen to increase in size as the cells synthesized proteoglycan, which became trapped in the collagen matrix and attracted water into the structure. The chondrocyte production of proteoglycan was verified histologically. The deformations to the structure changed in nature from plastic (instantaneous modulus ~0.4-0.5 MPa) to poroelastic (instantaneous modulus ~0.5-0.7 MPa after 10 days culture) with the increasing water storage. Elastographically, the elastic properties of the engineered samples were seen to be reasonably depth-independent.

IV Discussions

The global mechanical stress-relaxation results demonstrate the poroelastic nature of both the nasal and the articular cartilage, in that the stress required to maintain the constant deformations decreases with respect to time. Articular cartilage additionally showed strain dependent instantaneous elastic moduli which were absent in the nasal cartilage results. This significantly highlights the importance of the contribution of the fibril orientation to the global mechanical behaviour of the tissue. The equilibrium moduli of the two hyaline cartilage structures were very similar, which demonstrates that although very important at the instant of loading, the collagen orientation has little effect once stress-relaxation has occurred. The mechanical tests in themselves did not provide information regarding localised deformations, but provided a reference for comparison with the depth-dependent moduli at any given test time.

Evidence of layering was seen in the articular cartilage samples from the strain maps obtained using the elastography technique. The nasal cartilage strain maps followed approximately linear trends. Statistically (paired t-test >0.5) there was no significant difference between the strains in the top or middle of the nasal specimens. The noise in the nasal data was possibly caused by localised cell concentration variations depending on the site of sample extraction on the septum as chondrocytes have a considerably lower [4] elastic modulus than the surrounding matrix. The articular cartilage strain maps demonstrated a sharp increase about two thirds from the base of the structure. The tissue depths shown are relative to the ultrasonic transducer, which is at the base of the specimen. The steeper gradient of strain versus depth implies a lower stiffness than the other regions, which is consistent with theory. The differences between the superficial, middle and deeper regions were found to be statistically significant (paired t-test <0.05), with the larger differences at lower strain levels. The shapes of the strain maps, particularly for articular cartilage, also appear to be dependent on the global applied strain. Strain dependent deformation mechanisms are most likely designed to protect lower tissue regions. At the lower applied strains, the middle zone of articular cartilage appeared less stiff than at the higher levels of strain. These strain map results were expected due to the differences in elastic properties of the regions within the articular cartilage and the more uniform nature of the elastic properties in the nasal cartilage. The strain maps were also seen to demonstrate slight dependence on time, indicating a change in the internal structure of the samples during the relaxation period. This effect was present in both the nasal and the articular cartilage tissue. As the samples underwent poroelastic relaxation, the large stress concentrations, responsible for the regions of high strain, were redistributed into the adjacent tissue, causing a certain degree of equilibration of the strain levels. Changes in the speed of the propagating sound were determined to be negligible in affecting the signal arrival time from the results of a mathematical assessment.

The engineered tissue constructs used in this experiment represent a preliminary form of synthetic cartilage tissue, which were simple in construction. The samples at the first developmental stage had instantaneous global elastic modulus properties slightly less than those of the simple collagen construct (~0.4-0.5MPa); the slight reduction was most likely due to the presence of the chondrocytes. After both five, seven and ten days of tissue culture, the increase in the construct global elastic modulus (1%, 3% and 6% respectively) and time taken to reach equilibrium was a result of the chondrocyte expression of the collagen and proteoglycan matrix. As the proteoglycan concentration increased, the tissue absorbed water and increased in thickness. The proteoglycans also assisted construct recovery when the load was removed. The tests on the engineered tissue samples demonstrated important benefits of elastography. Ultrasound elastography can be used to detect small changes in this engineered tissue and therefore would be suitable for testing the tissues throughout their synthesis. Small variations in regional elastic properties can be observed in some of these 'homogenous' samples. These small variations are likely to be caused by small gravitational effects impacting local concentrations of cells and collagen in the construct matrix during manufacture. There are important implications here for the tissue engineering of articular cartilage as the layering property is not trivial to the global mechanical behaviour.

V Conclusions

The results from elastography experiments demonstrate the ability to determine the depth-dependent behaviour of articular cartilage under load. The resolution of the elastography system was suitable for determining the elastic properties of engineered cartilage specimens. Ultrasound elastography was found to be a suitable technique for use in the design of engineered tissue properties and the *in vitro* monitoring of engineered constructs for implanting suitability.

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